



Fear and stress measurements in pure and hybrid red-legged partridges



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ABSTRACT

The purposes of this study were to examine the duration of induced tonic immobility (a reliable fear indicator considered a passive anti-predator behavioural trait) and two stress indicators (heterophil to lymphocyte ratio, and fluctuating asymmetry of leg length, middle-toe length, and wing length) in females and males of the red-legged partridge, and to analyze the differences between pure and hybrid populations. A total of 132 birds (66 pure and 66 hybrid partridges; 48 females and 84 males) at 52 weeks of age were used. There was a significant difference between pure and hybrid birds in tonic immobility duration ($P < 0.005$) and heterophil to lymphocyte ratio ($P < 0.02$), the duration being longer and the ratio being higher in the pure group of birds. There was a significant difference between pure and hybrid birds in the relative fluctuating asymmetry of middle toe length ($P < 0.002$) and the combined fluctuating asymmetry value of the three traits ($P < 0.03$). There were no significant differences between sexes in any indicator of fear or stress. Thus, pure red-legged partridges were more fearful, stressed, and asymmetrical than hybrid partridges, having increased tonic immobility duration, heterophil to lymphocyte ratio, and relative fluctuating asymmetry. Results indicate that levels of fear and stress, e.g. tonic immobility duration, heterophil to lymphocyte ratio, and fluctuating asymmetry, are higher in the pure partridge, and that fear and stress indicators have been modified in hybrid partridges leading to more passive animals.

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1. Introduction

The red-legged partridge (*Alectoris rufa*) is a wild species native to Spain, whose pure populations have been decreasing due to the deterioration of their natural habitats and the increased hunting pressure. One major concern in game farms that breed birds in captivity has been the

genetic introgression with the aloctone species *Alectoris chukar*. Although *A. rufa* is the most appreciated of the hunted partridge species, the hybrid partridges are preferred for the commercial hunting farms because they are heavier birds, with increased laying period and egg production (Gaudioso et al., 2002). However, the hybridization and the intensive production have also induced considerable behavioural and physiological modifications, such as a reduced anti-predator behaviour (Alonso et al., 2005; Pérez et al., 2010). This loss of anti-predator behaviour is mainly attributed to habituation to humans (Csermely

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et al., 1983), isolation from predators (Gaudioso et al., 2011), and absence of parents or fosters during rearing (Sánchez-García et al., 2011).

There are no previous studies on three frequently used indicators of fear and stress: duration of tonic immobility, heterophil to lymphocyte ratio, and fluctuating asymmetry in the red-legged partridge, although a study has analyzed a different indicator of fearfulness: the emergence test (Pérez et al., 2010), which is assumed to be a mildly fear-evoking and less reliable test than the tonic immobility duration is (Forkman et al., 2007). These authors studied the escape response of red-legged partridges captured in the wild and from a commercial hunting farm, using as fear indicator the latency to escape from a cage (the lapse of time between the moment the release cage was opened and the moment in which the partridge started the escape). The tonic immobility reaction is a well established test of fear (Gallup, 1979; Forkman et al., 2007), assumed to originally have developed as a passive defence reaction facing a direct predator attack, deceiving the predator into believing the prey is already dead (an alternative behaviour to “fight or flight” response). Although, the most common animal response to fear or threat is the “fight or flight” response, many vertebrates and invertebrates animals attacked by predators often enter a state of tonic immobility in which individuals appear to simulate death, remaining motionless. Many species show induced tonic immobility in response to physical restraint (Ferreira et al., 2012), and it is often used in domesticated animals (poultry, pigs, and rabbits) to evaluate fearfulness related to coping with predation (the longer the more fearful). Jones and Satterlee (1996) indicated that fearful quail are likely to adopt a passive coping strategy (immobility) for responding to threat, whereas less fearful quail more closely resemble active (fight/flight) copers. Similarly, Erhard et al. (1999) proposed that tonic immobility is one possible way of assessing whether pigs are more likely to adopt a more active (short duration of tonic immobility) or a more passive behavioural strategy (long duration of tonic immobility) in a challenging situation. Edelaar et al. (2012) suggested that tonic immobility can be interpreted in a context of boldness toward predators, making it a practical behavioural trait for studies involving anti-predation behaviour. Thompson et al. (1981) found that cats choose an active quail more often than a quail in tonic immobility, suggesting that tonic immobility eliminates the movement stimuli that sustain further attack. Madden and Whiteside (2014) indicated that pheasants that survived the hunting season were more fearful. Binazzi et al. (2011) suggested that freezing was the most frequent anti-predator behaviour in the red-legged partridge following the appearance of an aerial predator, whereas escape was more frequent with a terrestrial predator.

Furthermore, birds that show greater fear responses may have greater physiological responses to stress. Fear is a powerful stressor which can seriously damage the welfare of animals (Jones, 1996), and reduced fear may be accompanied by a generally lower stress sensitivity. Jones (1989) suggested that heterophil to lymphocyte ratio may be greater in fearful Leghorn pullets than in less fearful birds, and Calandrea et al. (2011) found that higher fearfulness potentiates the effects of stress in the Japanese quail. Stress

in chickens leads to increase production and release of corticosterone and to immunosuppression (Siegel, 1995), causing higher heterophil to lymphocyte ratio (Maxwell, 1993). The heterophil to lymphocyte ratio is considered as a good indicator of chronic stress in poultry (the higher the more stressful; Gross and Siegel, 1983) and other vertebrates (Davis et al., 2008). Hall et al. (2014) showed that restraint induced chronic psychological stress induces a decrease in lymphocytes (increasing heterophil to lymphocyte ratio). Moreover, the fluctuating asymmetry, defined as the small and random deviations from the perfect symmetry caused by environmental or genetic stress during the development (Van Valen, 1962), is commonly considered as a developmental stability estimator, and can be used as a historical measure of stress although would not reflect the response to current conditions (Møller and Swaddle, 1997; Tuytens, 2003; Knierim et al., 2007).

The purposes of this study were to examine the duration of induced tonic immobility (a reliable fear indicator considered a passive anti-predator behavioural trait) and two stress indicators (heterophil to lymphocyte ratio, and fluctuating asymmetry) in the red-legged partridge at 52 weeks of age, and to analyze the differences between pure and hybrid populations. Both females and males (at 52 week of age) were included in the study in order to investigate any sex differences in fear and stress indicators, because responses in females can be different than in males, and these sexually differentiated responses appear early in development, becoming larger at juvenile and adult ages, and abating at advanced ages (Campo and Carnicer, 1993; Campo and Dávila, 2002; Campo et al., 2005; Luine et al., 2007). As far as we know, the use of these fear and stress indicators has not been studied yet in red-legged partridges. We predicted that the anti-predator behaviour in this species has been modified due to selection for intensive production and genetic introgression. The hybrid partridges were expected to have shorter duration of tonic immobility, lower heterophil to lymphocyte ratio, and lower fluctuating asymmetry than the pure red-legged partridges.

2. Materials and methods

All procedures were approved by the Institutional Animal Care and Use Committee of the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain.

2.1. Birds and experimental design

The birds used in this study were 66 *A. rufa* pure red-legged partridges obtained from the Red-legged Partridge Station of Lugar Nuevo (Andújar, Spain). This Station belongs to the Andalusian Regional Government, and maintains currently a pure red-legged partridge population without any genetic introgression (Dávila, 2009). Sixty six additional hybrid partridges, showing genetic introgression from *A. chukar* (>25%), were obtained from a commercial farm located in Segovia (Spain). The genetic introgression shown by these two populations were tested according to official genetic

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